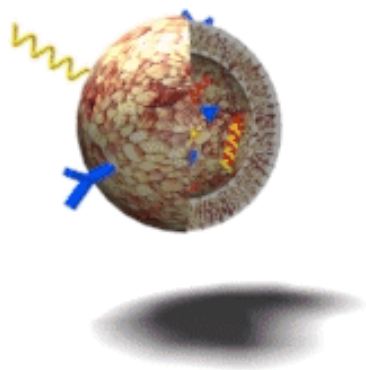


PRACTICAL APPROACHES FOR DISSOLUTION TESTING OF NANO FORMULATIONS



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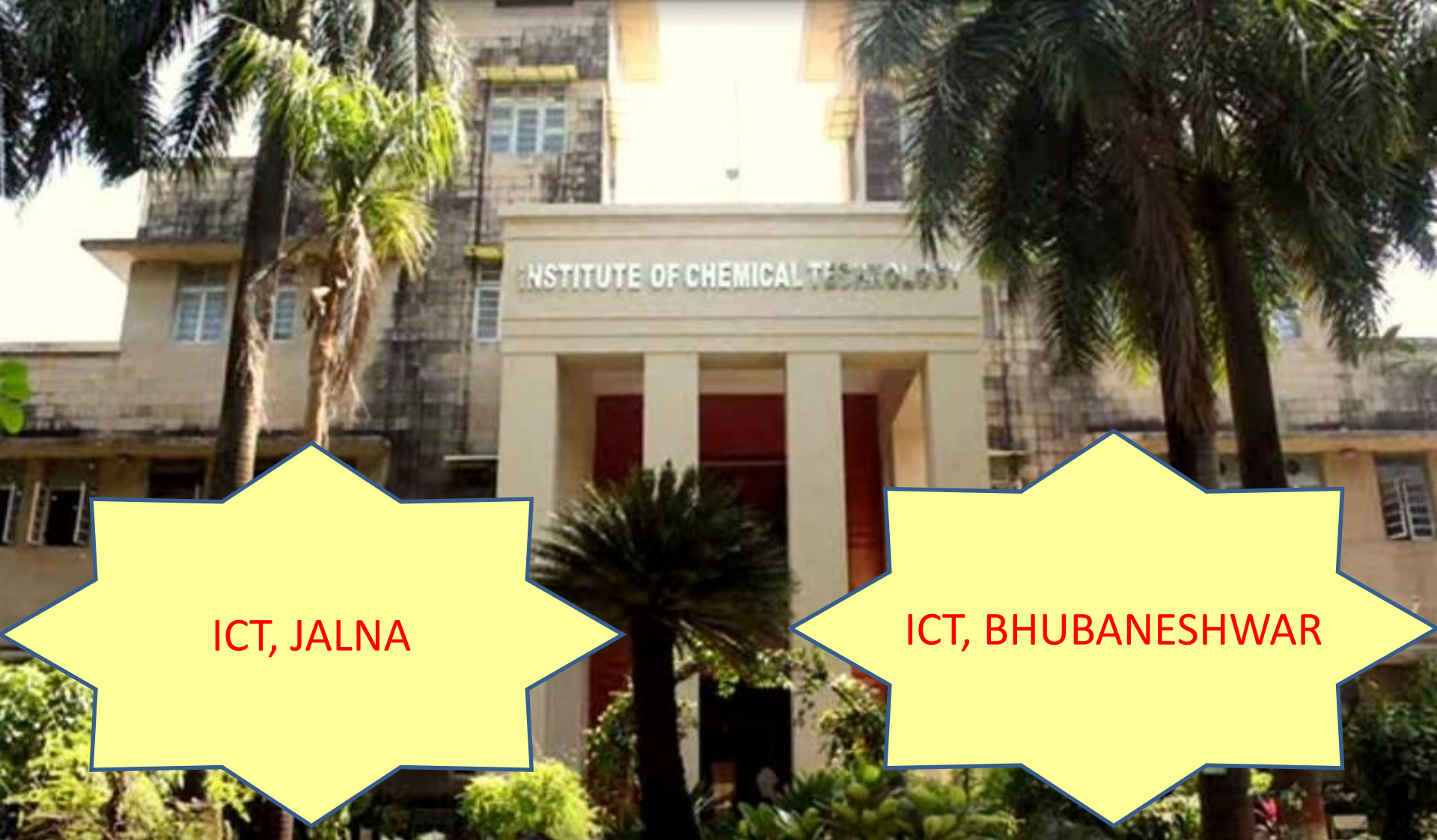
Institute of Chemical Technology (ICT)

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E-mail: pvdevarajan@gmail.com

DISSO INDIA 2019- CHANDIGARH SEPTEMBER 12-13, 2019



ICT, JALNA

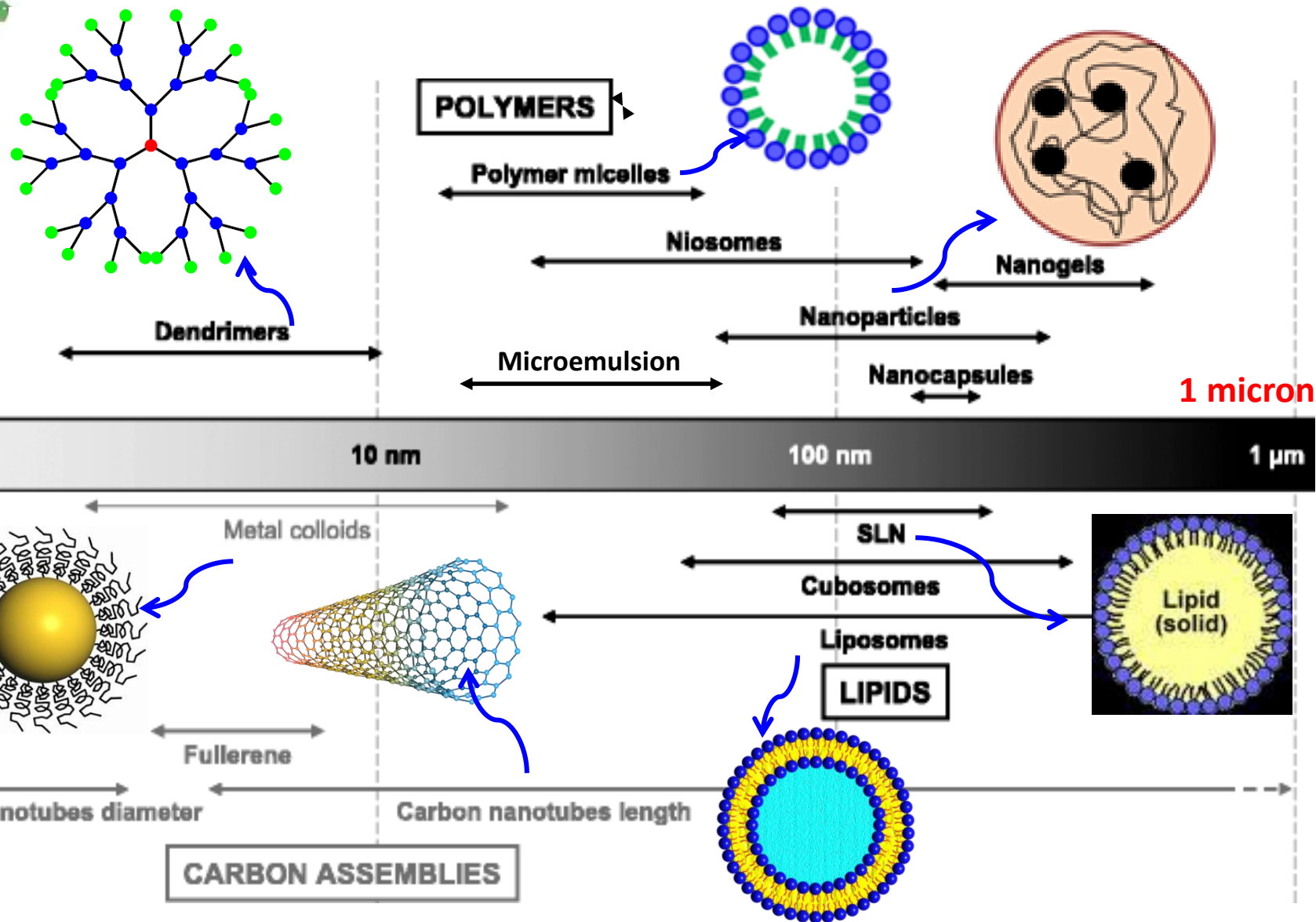
ICT, BHUBANESHWAR

Institute of Chemical Technology (ICT)

**Deemed University, Elite status and Centre of Excellence (GOM),
Mumbai 400 019, INDIA**



NANOCARRIERS IN NANOMEDICINE





NANOMEDICINE SUCCESS STORIES



ABRAXANE



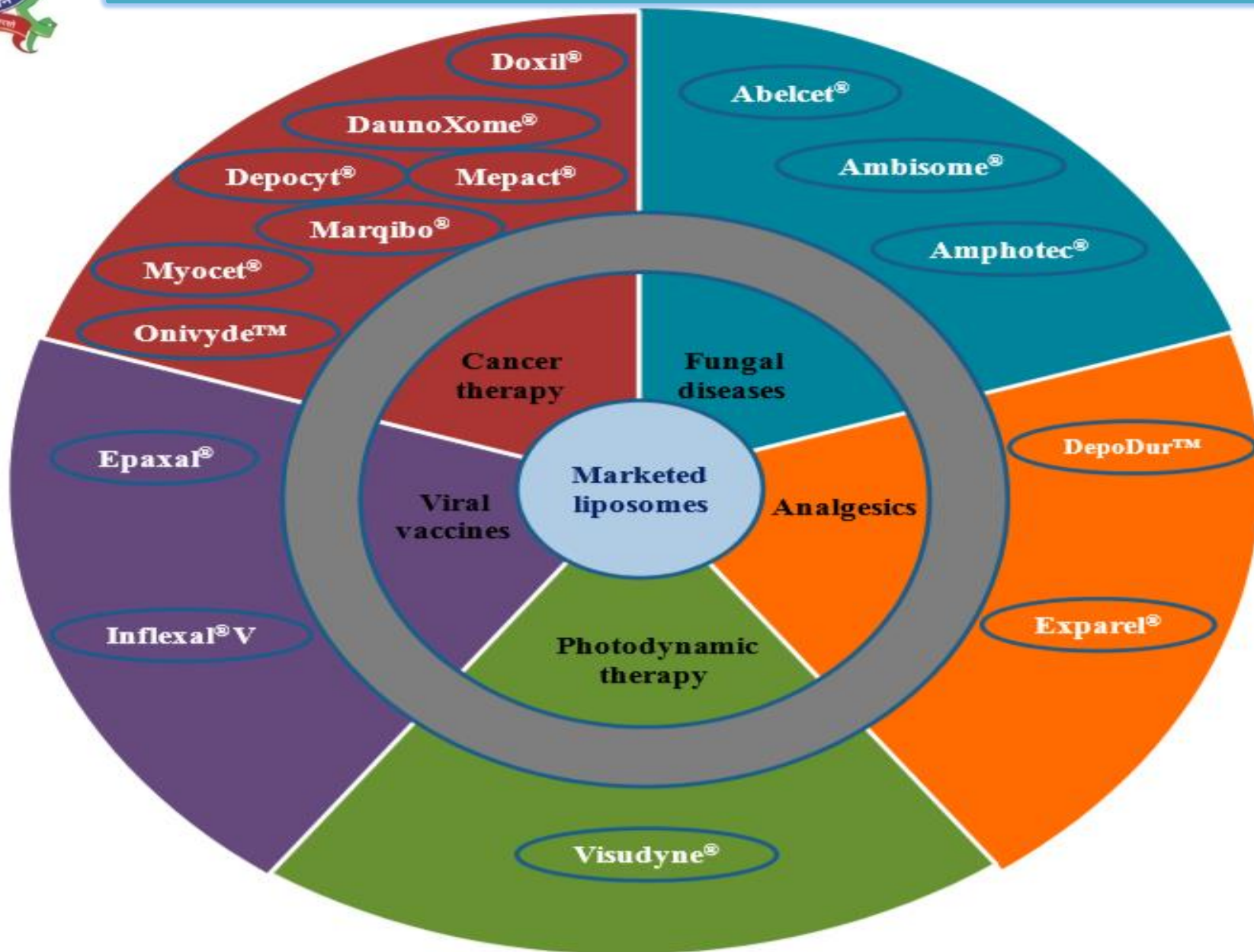
DOXIL

AMPHOTERICIN



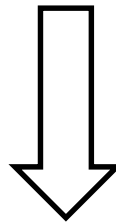


NANOMEDICINE SUCCESS STORIES

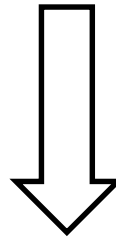


Nanosystems and Need for *in vitro* Dissolution testing

Nanosystems are promising



Unavailability of standardized *in vitro* dissolution method



Urgent Need to develop Standardized Testing Methods



CHALLENGES IN DISSOLUTION TESTING OF NANOMEDICINES

SMALL SIZE

**Difficulty in Separation of NP
from medium**

COMPLEX SYSTEM

Complexity of System type

Target specific release

Programmed Release

MEDIA SELECTION

Biorelevant dissolution media

Environment specific release (pH, temp)

Sink conditions



IDEAL DISSOLUTION TEST

- **REPRODUCIBLE**
- **ROBUST**
- **PHYSIOLOGICALLY RELEVANT MEDIA**
- **DISCRIMINATORY**
- **CONVENIENT**
- **EASY TO USE**
- **MAINTAIN SINK CONDITION**
- **ADAPTABLE TO MANY FORMULATIONS**



IN VITRO DISSOLUTION TESTING METHODS FOR NANOMEDICINES

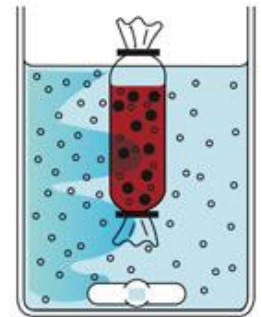


DISSOLUTION METHODS

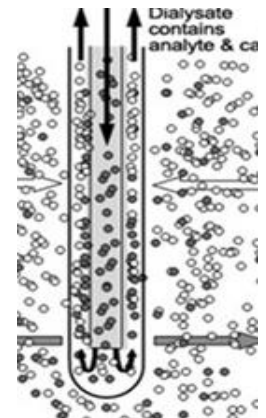
❑ Sample & Separation methods



❑ Membrane Diffusion (Dialysis Sac) methods



❑ Others (Micro dialysis, Dynamic dissolution & 2 stage reverse dialysis)



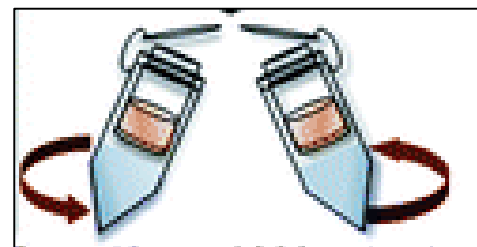


SAMPLE & SEPARATION METHODS

- NP directly added in medium & separation techniques applied
- Drug content in supernatant or filtrate is analyzed



Ultrafiltration



Ultracentrifugation



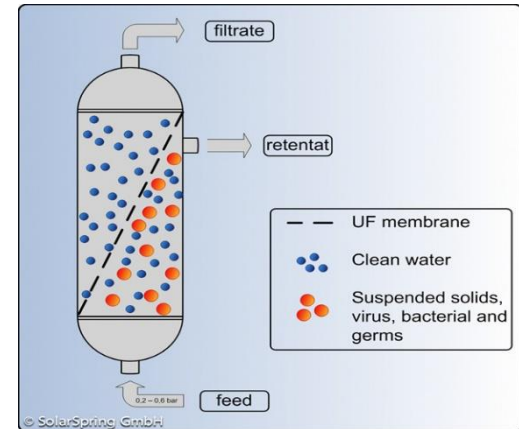
Key parameters : **Sample separation technique**
Agitation conditions



SAMPLE & SEPARATION METHODS

Pressure Ultrafiltration

- Completely separate Nanoparticles from release media within 5 min
- Prevent Clogging of filter pores



SCHEMATIC

Syringe Filtration

- Use of Syringe filters with smaller pore size (0.1 to 0.02 μm) has been used

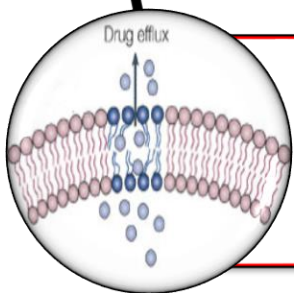




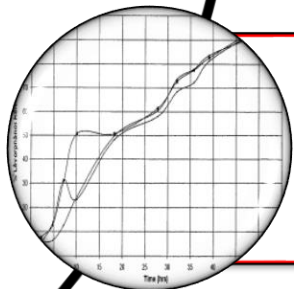
DISADVANTAGES OF SAMPLE & SEPARATION METHODS



Difficulty in separation of NP from media though high external energy applied



Long-time & High speed can result in destabilization of system (e. g. Nanoemulsion & Liposome)

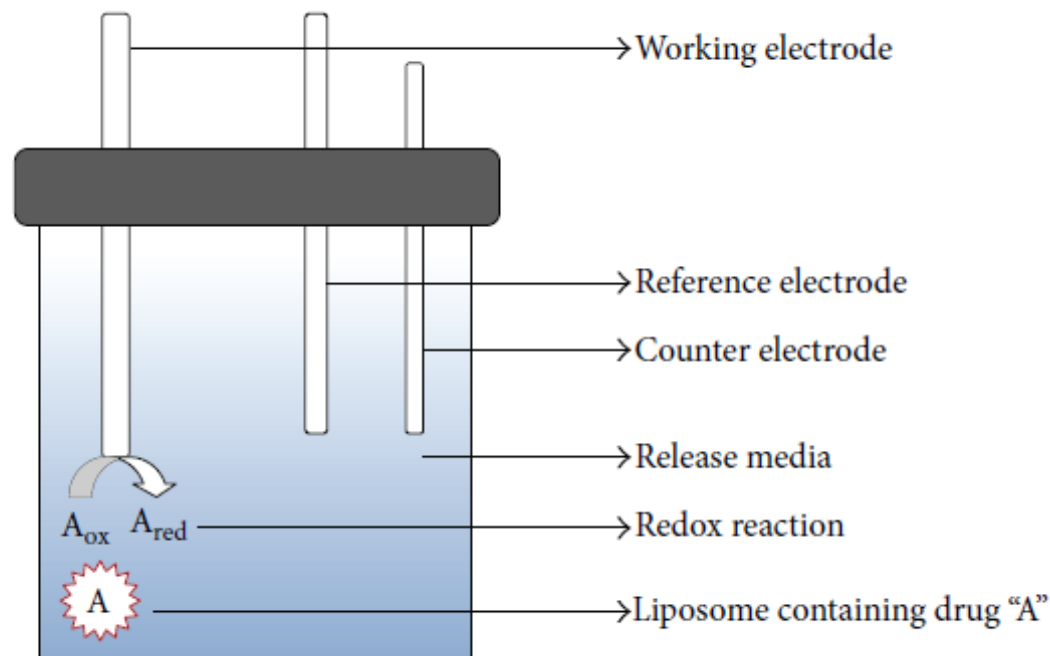


Drug release continues during separation process, which can lead to erroneous results



ADVANCED SAMPLE AND SEPARATION METHOD

DYNAMIC DISSOLUTION



Utilize ion- or drug-selective electrodes to monitor the dissolution/release profiles of electroactive drugs

Not suitable for non-electroactive drugs



DIALYSIS METHODS

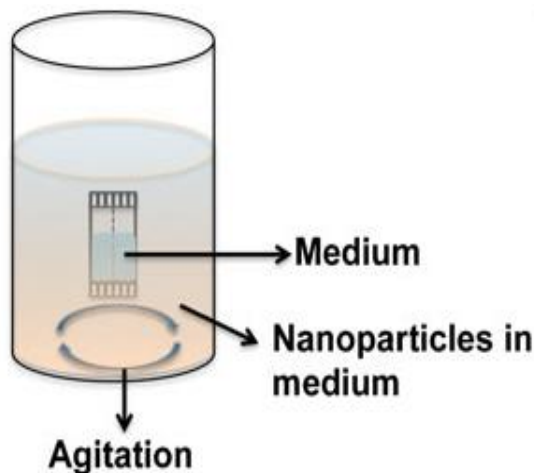


MEMBRANE DIFFUSION METHODS (DIALYSIS BAG)

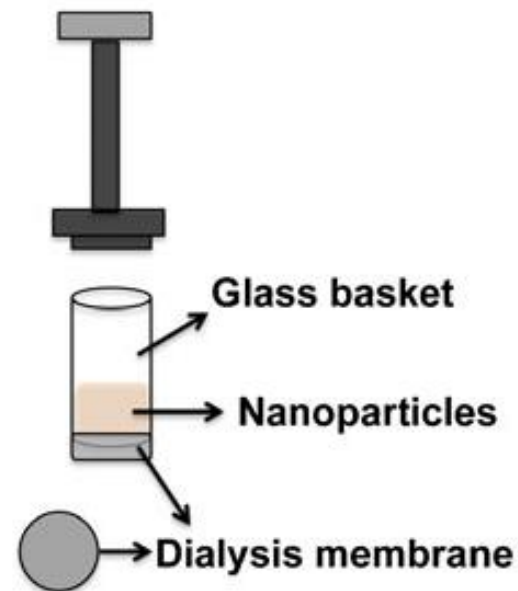
Nanosystems separated from the release medium through dialysis membranes that are permeable to the free drug but impermeable to the nanosystems



Dialysis Sac Method



Reverse Dialysis Sac Method

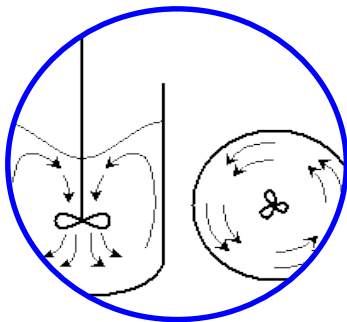


Side-by-Side-Dialysis



DIALYSIS METHODS

KEY PARAMETERS

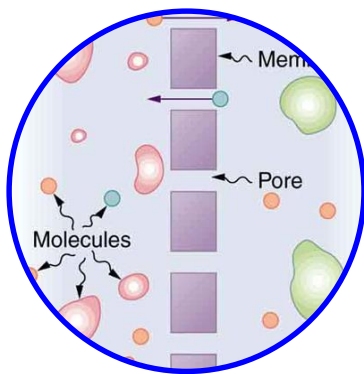


Agitation Conditions



Ratio between Donor & acceptor cell Volume

Inside Volume 6 to 10 fold less than medium volume



Molecular Weight Cut-Off (MWCO) of membrane

MWCO 100 times more than drug MW



DISADVANTAGES OF DIALYSIS METHODS

Lack of adequate agitation inside membrane

Absence of sink condition
(fixed volume)

Hindrance to drug diffusion through membrane

Reverse system causes high dilution of Nano system thus medium loses its discriminatory ability

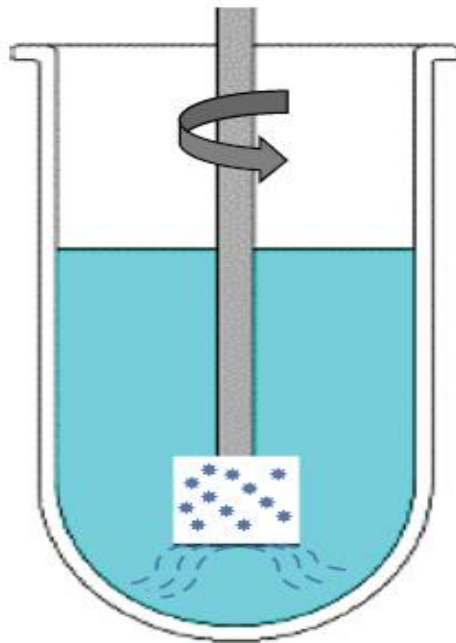
Disadvantages of Dialysis Methods



MODIFIED OFFICIAL APPARATUS

☐ Constant Volume

- USP I & II



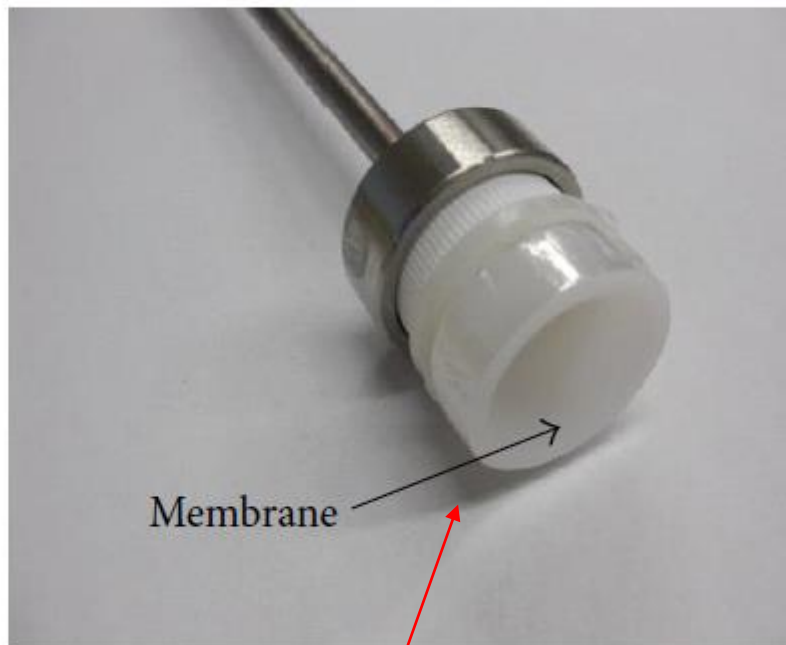
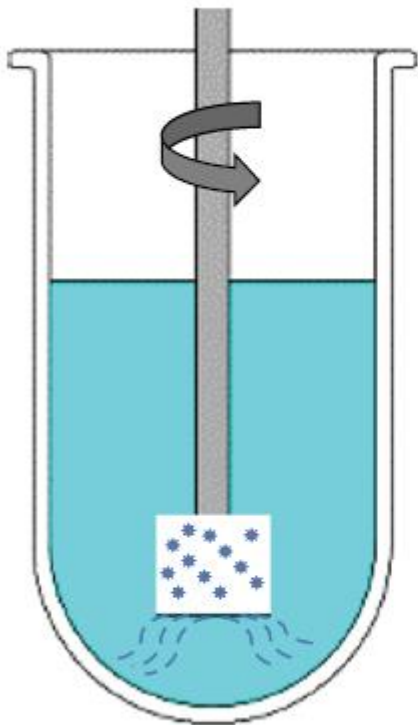
☐ Continuous Flow Methods

- USP IV





ADAPTATION OF DIALYSIS AND USP TYPE I

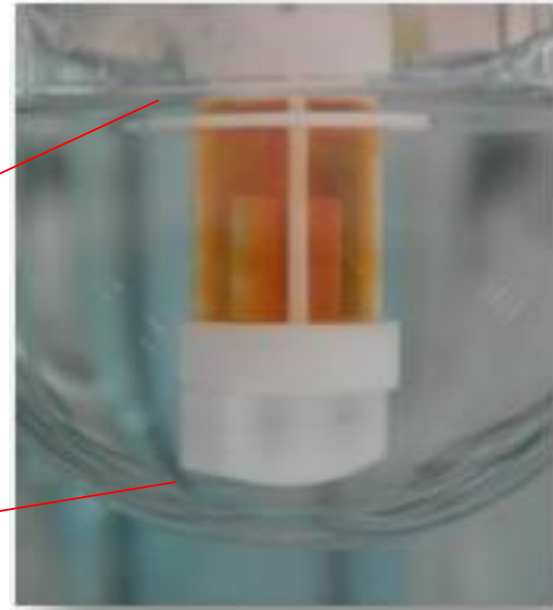


BASKET MODIFIED INTO A DIALYSIS CELL

Yuan Gao et al, BioMed Res. Inter., 2013



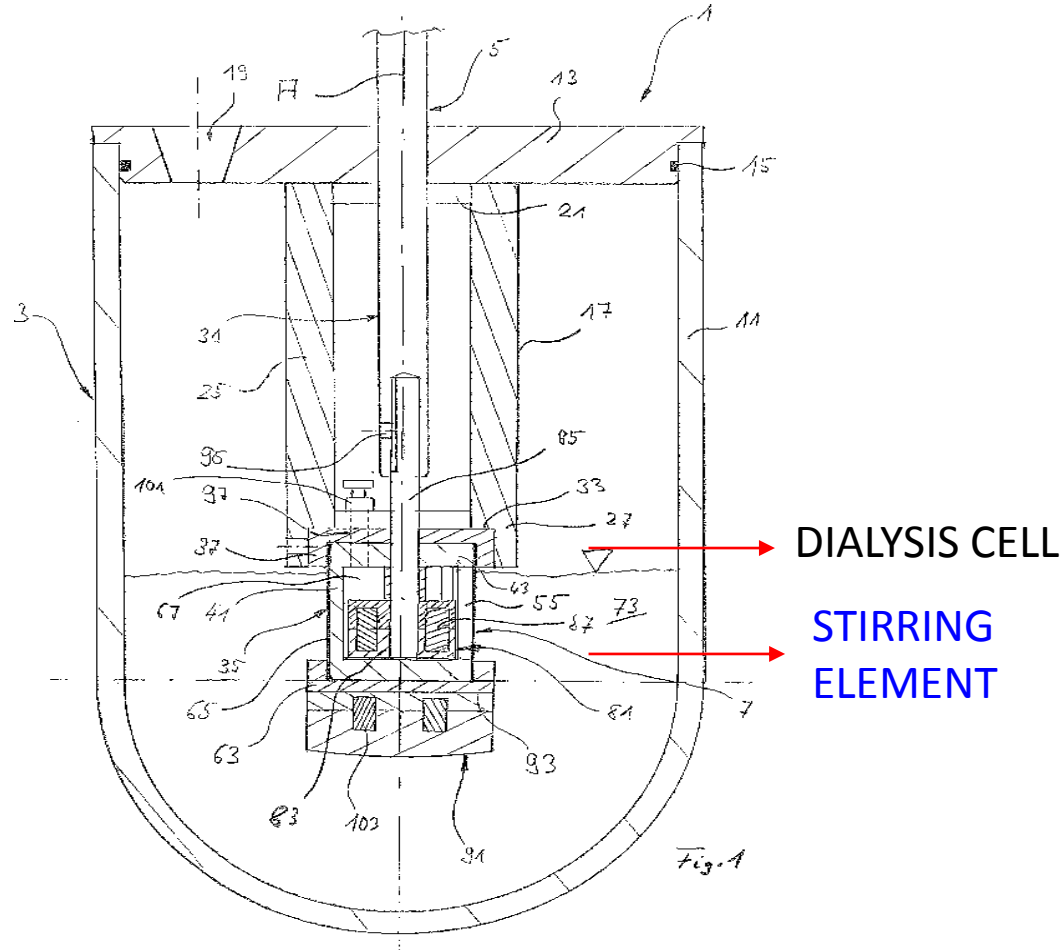
ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)



- Pharma Test offers the “dispersion releaser”
- High sensitivity for fluctuations in release rate
- Works well for compounds **with poor, moderate and good solubility**



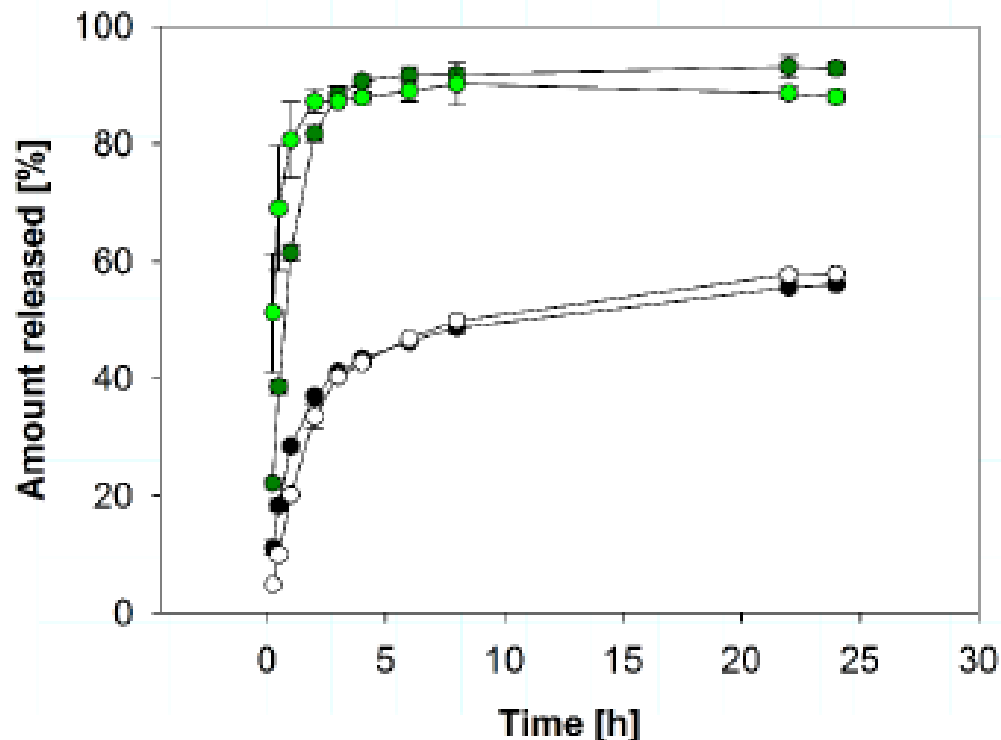
ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)



Patent No. DE102013015522.3



ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)



- Free drug, dispersion releaser
 - Free drug, dialysis bag
 - /○ SR formulation 1/2
- Setup USP2 / dialysis bag or dispersion releaser
- pH 7.2
- Medium phosphate buffer saline
 10% FCS

Batch-to-batch reproducibility high



CONTINUOUS FLOW THROUGH CELL TYPE IV

This method has been widely used to investigate drug release from microspheres

But Nanoparticulate systems have very small particle size ($<100\text{nm}$), challenging to test their release in USP IV.

CHALLENGE:

- NP clog the filter leading to slow flow rates and high pressure build-up in the system
- Pass through filters, thus resulting in erroneous data.

SOLUTION: Novel Dialysis Adaptor USP type IV

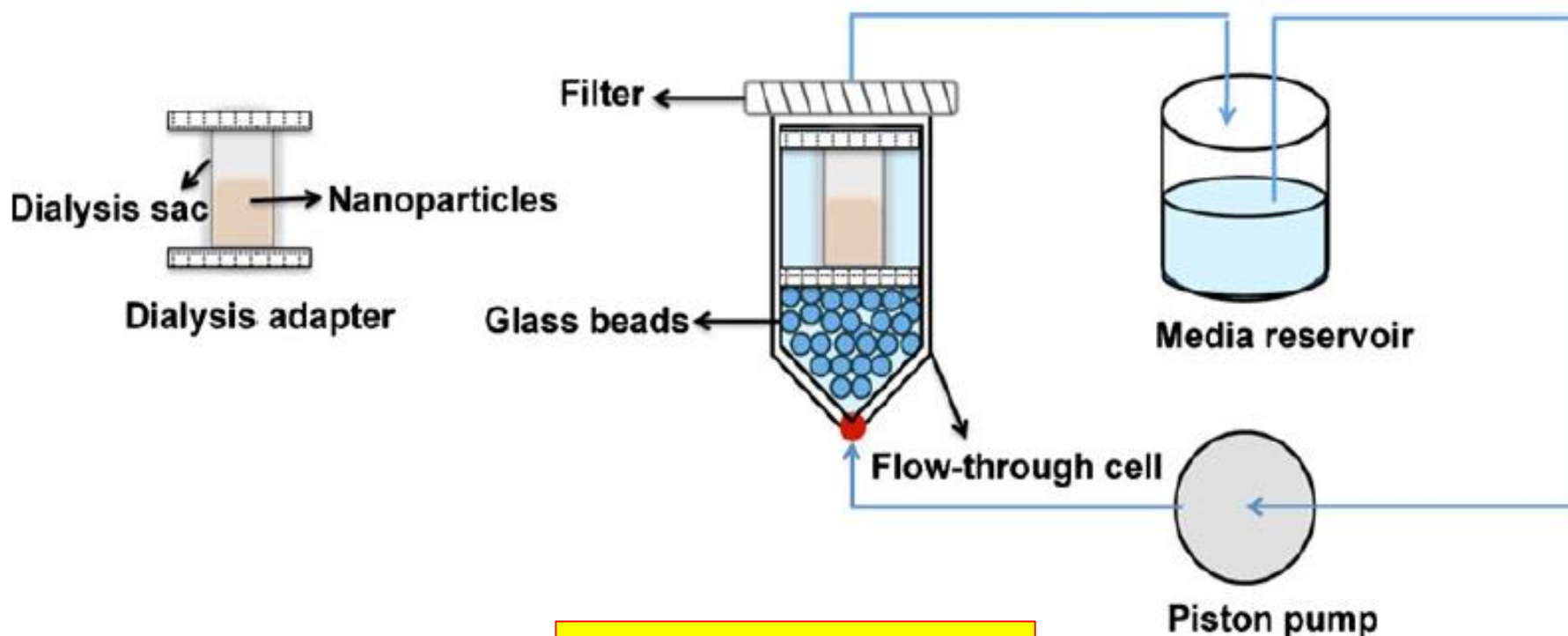


CONTINUOUS FLOW THROUGH CELL TYPE IV





CONTINUOUS FLOW THROUGH CELL TYPE IV



SCHEMATIC



CONTINUOUS FLOW THROUGH CELL TYPE IV - DIALYSIS CELL



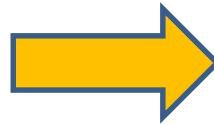
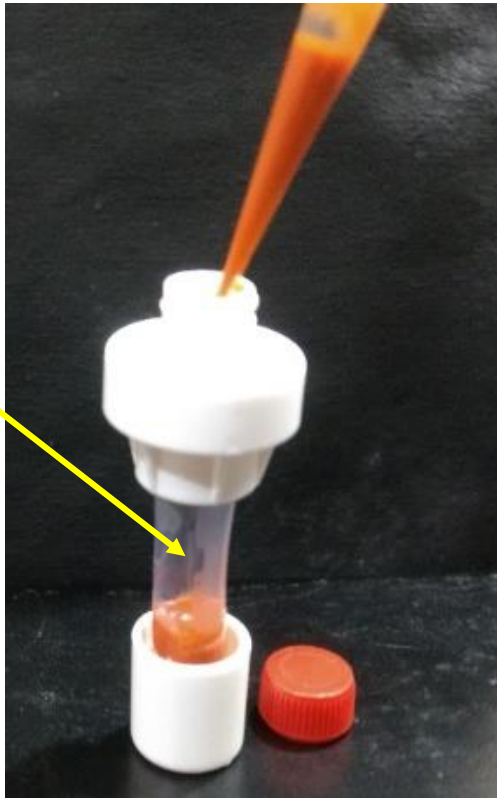
- ✓ High Discriminative power
- ✓ Avoided Filter clogging
- ✓ Avoided violation of sink conditions
- ✓ Avoided lack of agitation

Adaptor Tedious to handle



FLOAT-A-LYZER

Dialysis
Tubing



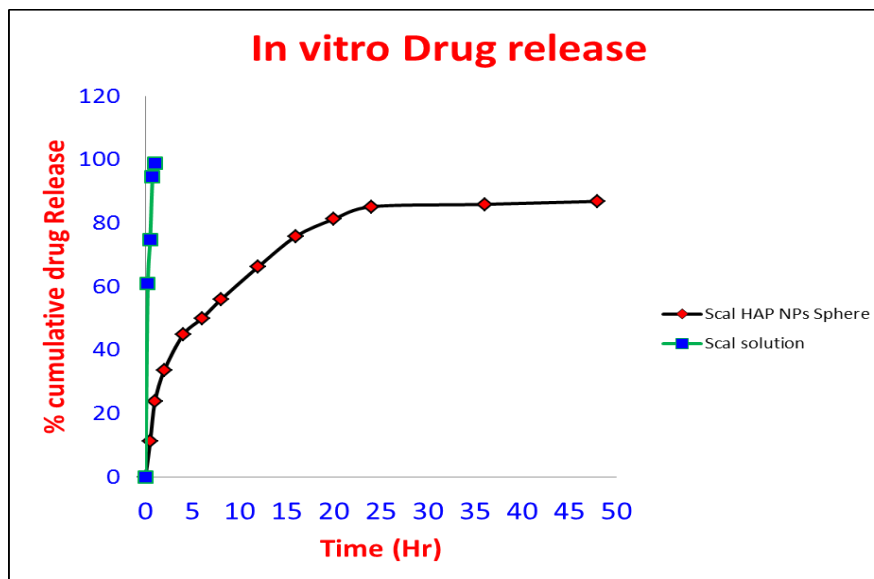


CASE STUDIES

USP APPARATUS IV WITH DIALYSIS CELL



INORGANIC NANOPARTICLES OF SALMON CALCITONIN USP IV



- 98 % of SCT high molecular weight drug (MW ~3000) in 1 hr indicates dialysis membrane not rate limiting
- **Sustained release seen with SCT NPs**



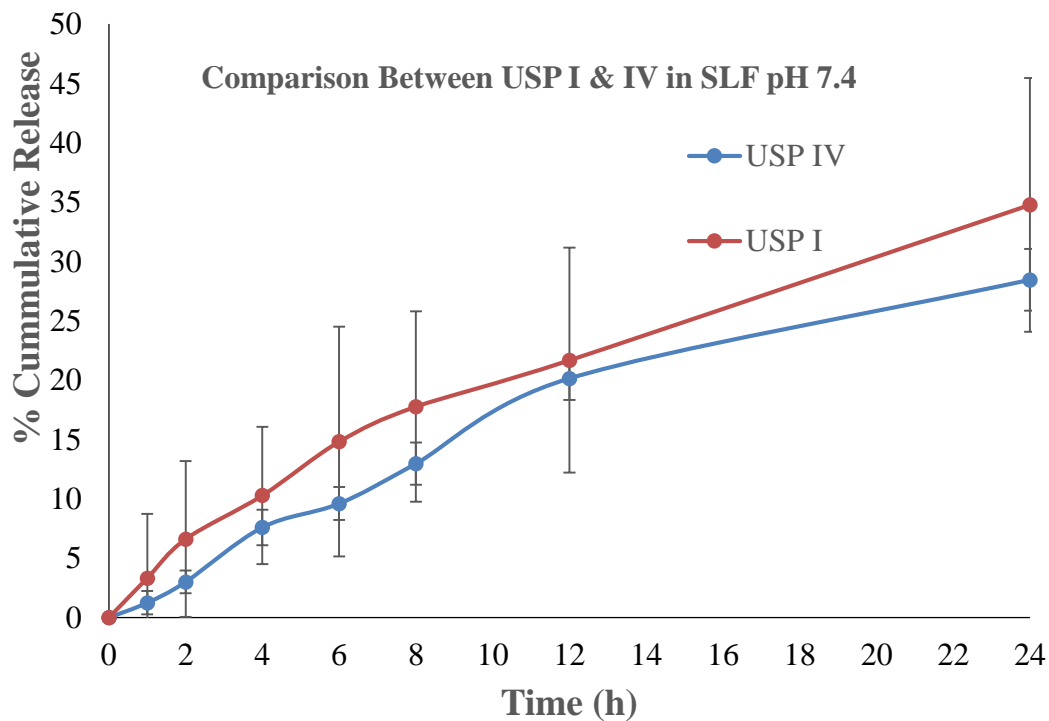
USP I VS USP IV

RIFAMPICIN MICROPARTICLES

USP I



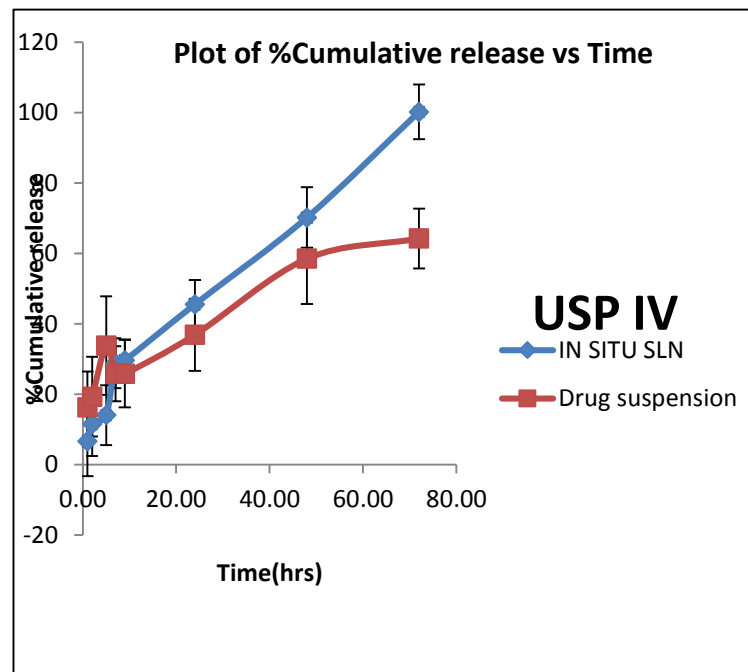
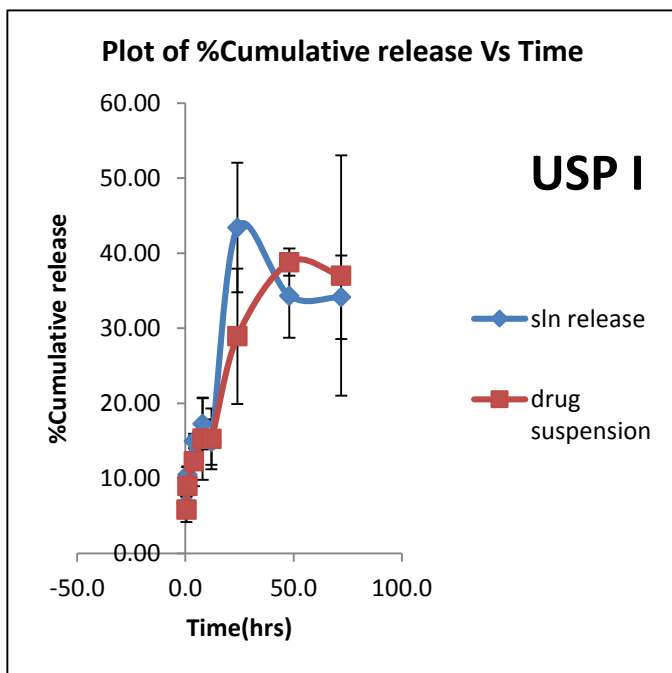
USP IV





USP TYPE I vs IV

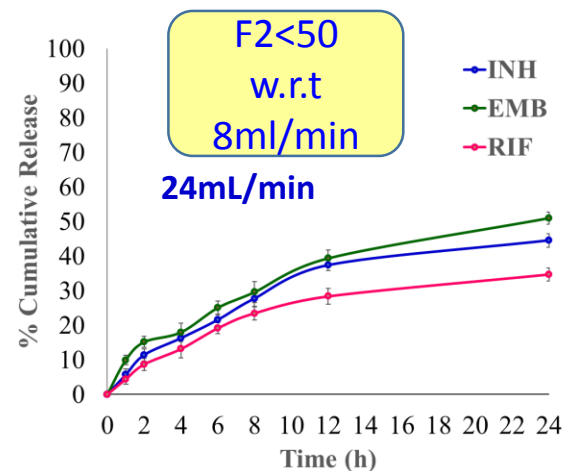
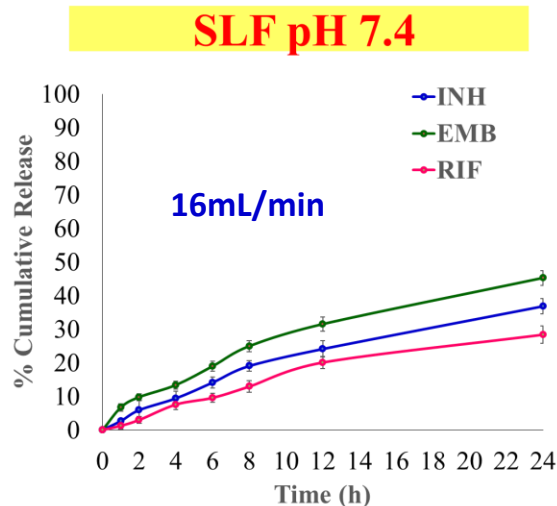
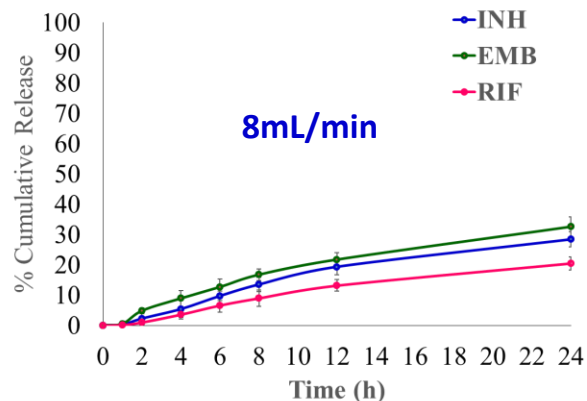
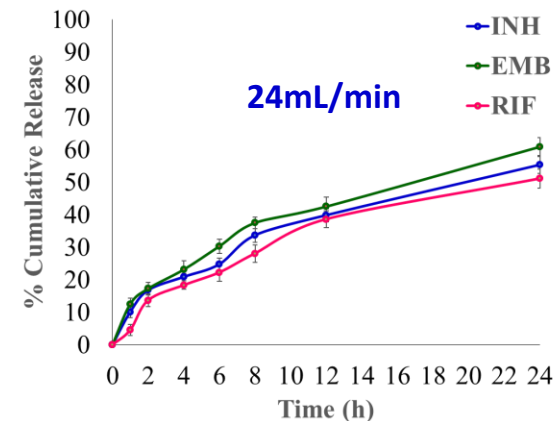
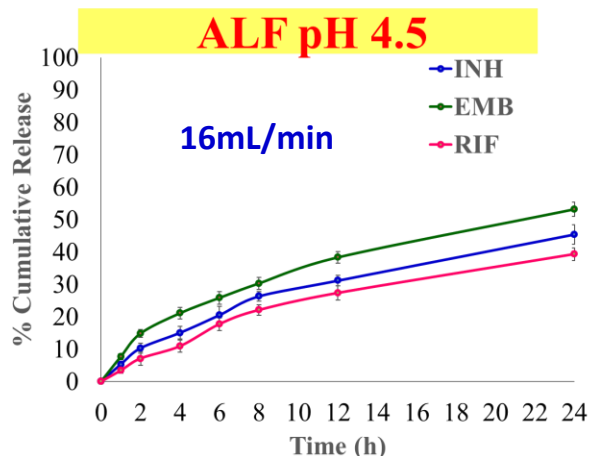
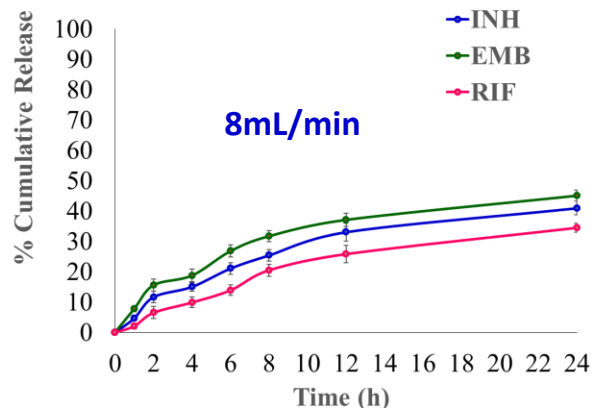
BUPARVAQUONE SLN



- **USP I – LOWER DRUG RELEASE DUE TO ABSENCE OF SINK CONDITION**
- **USP IV – COMPLETE RELEASE AND LOWER STANDARD DEVIATIONS**



EFFECT OF FLOW RATE & MEDIA IN USP IV

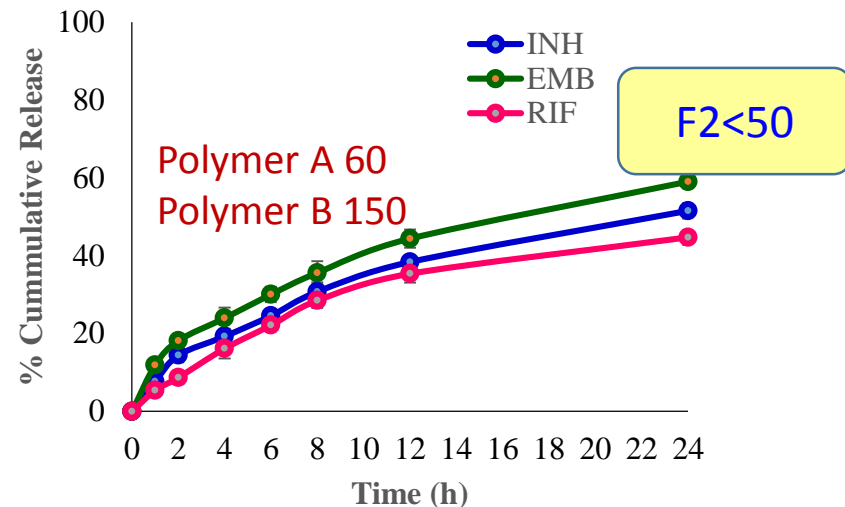
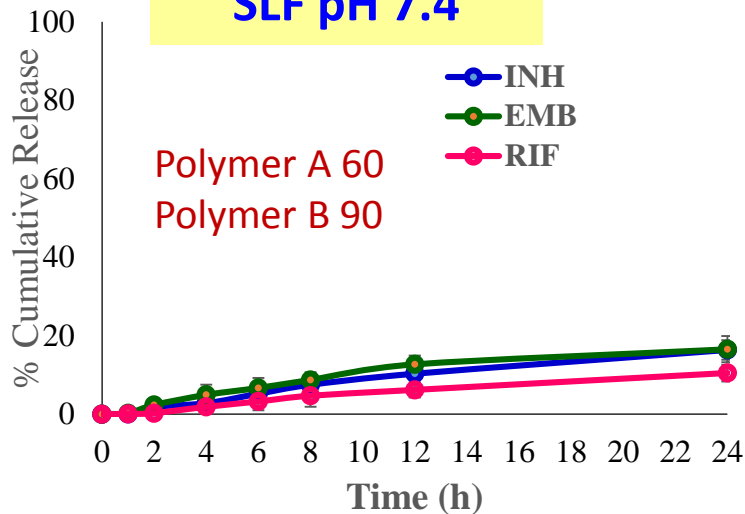


- Increase in Flow Rate 8-16mL - increase in amount dissolved
- Faster release in ARTIFICIAL LYSOSOMAL FLUID pH 4.5

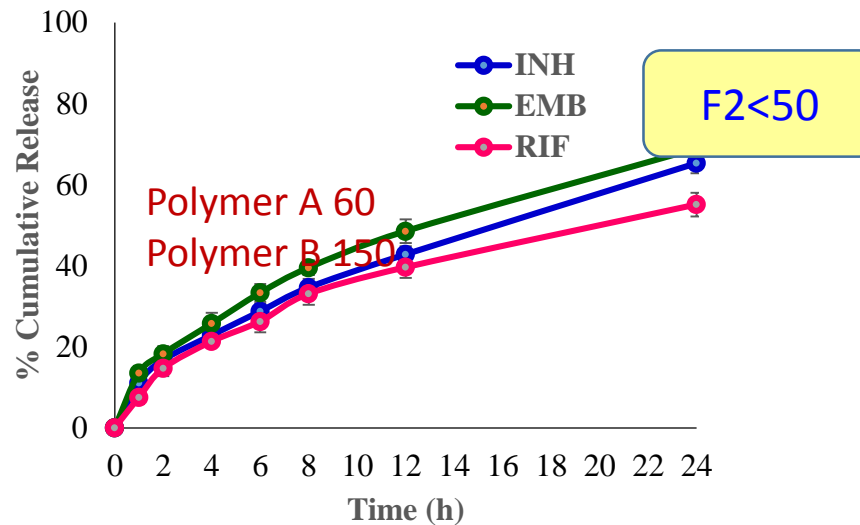
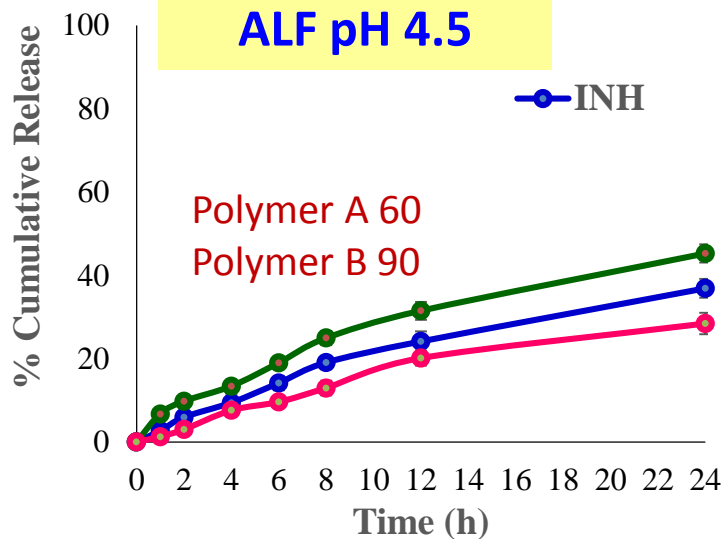


EFFECT OF FORMULATION COMPOSITION & MEDIA IN USP IV

SLF pH 7.4



ALF pH 4.5

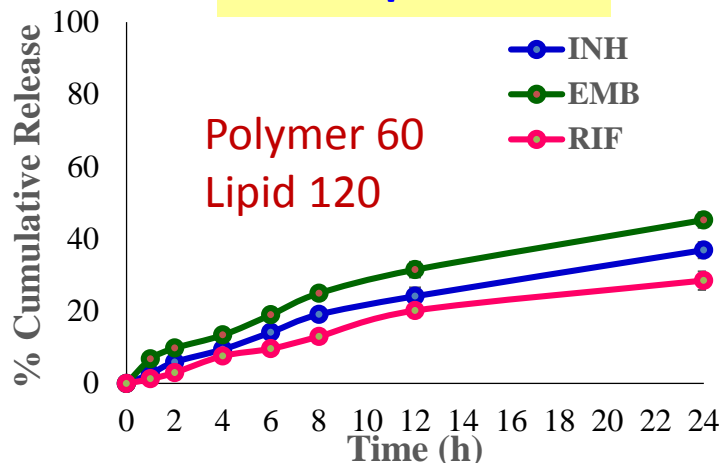


- Faster release in ARTIFICIAL LYSOSOMAL FLUID pH 4.5
- Increase in Polymer B increase in release rate

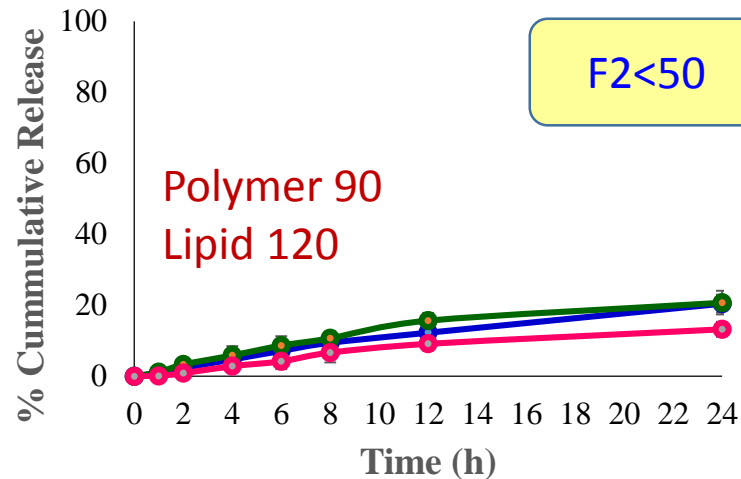


EFFECT OF POLYMER CONCENTRATION & MEDIA IN USP IV

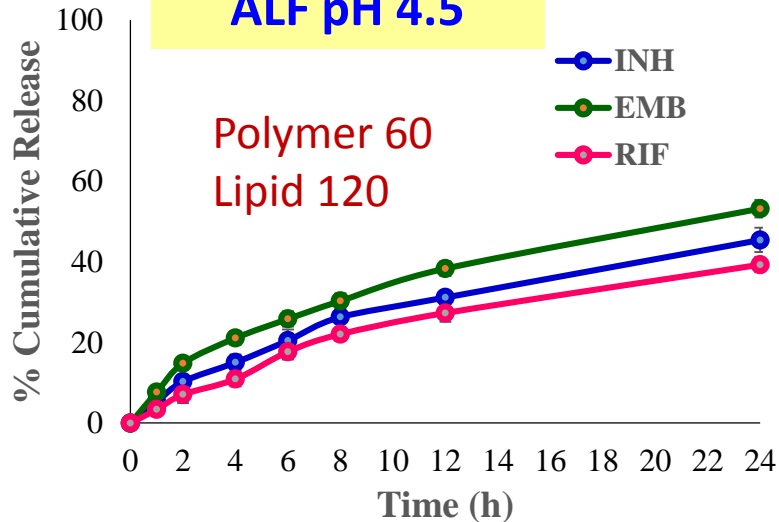
SLF pH 7.4



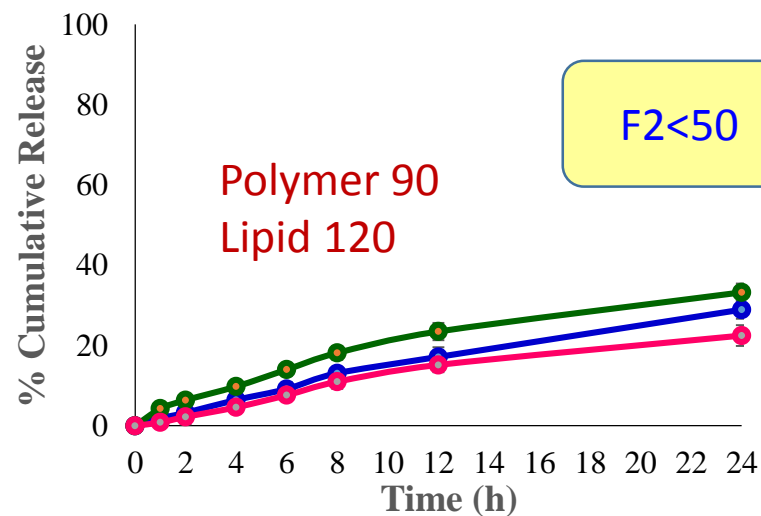
F2<50



ALF pH 4.5



F2<50



- Faster release in ARTIFICIAL LYSOSOMAL FLUID Ph 4.5
- Increase in Polymer decrease in release rate



DISCRIMINATORY DISSOLUTION AMPHOTERICIN B NANOSYSTEMS USP TYPE IV

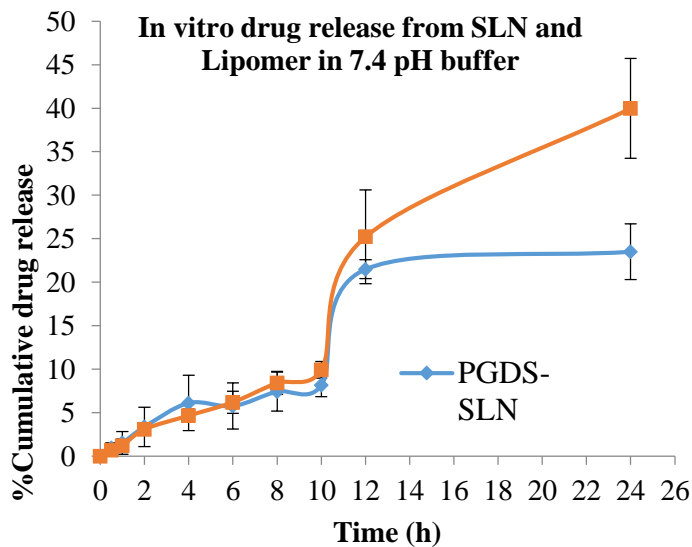
Volume of Media-100mL

Flow rate-6mL/min

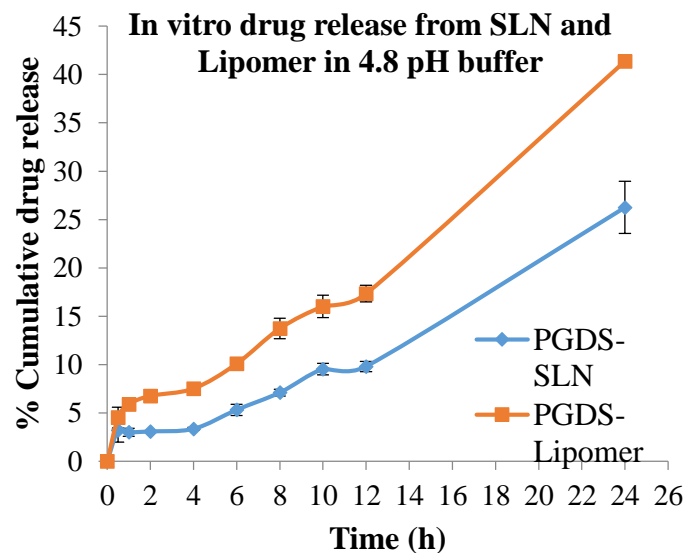
Sample volume-1mL

Aliquot volume-1mL

LIPOMER vs SLN



pH 7.4

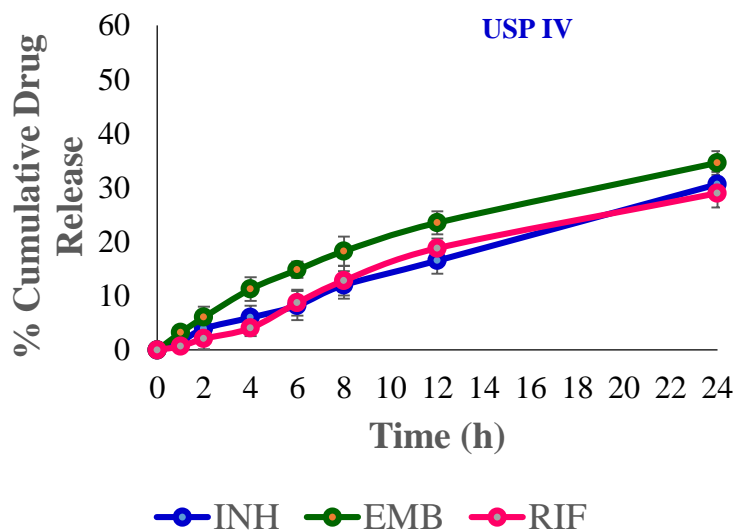
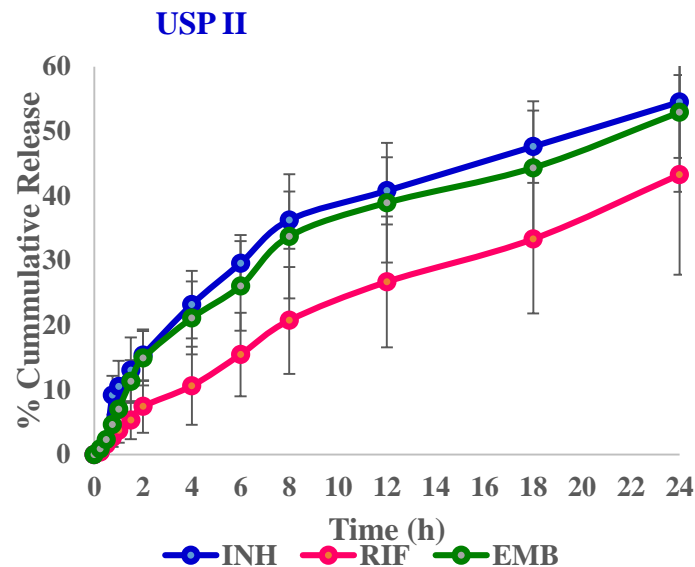
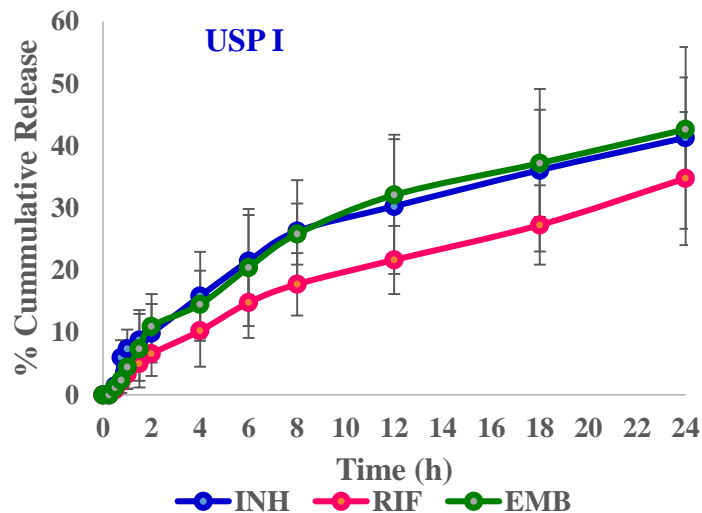


pH 4.5

- Discrimination evident at pH 4.8



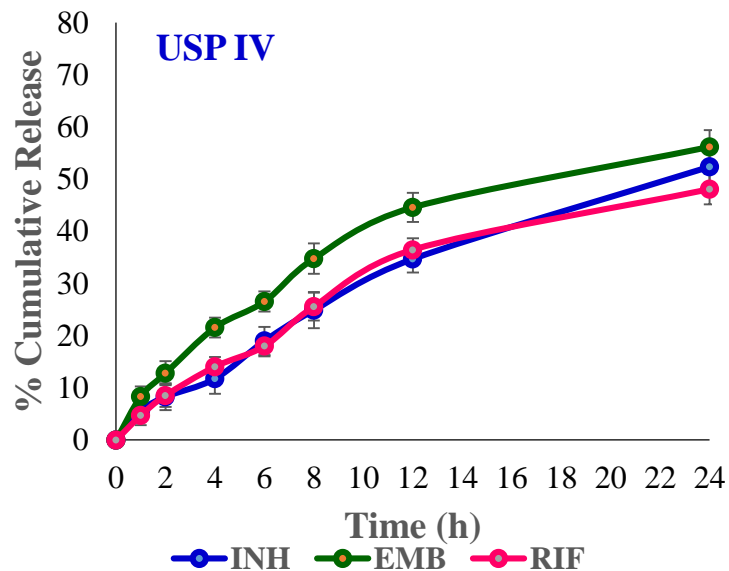
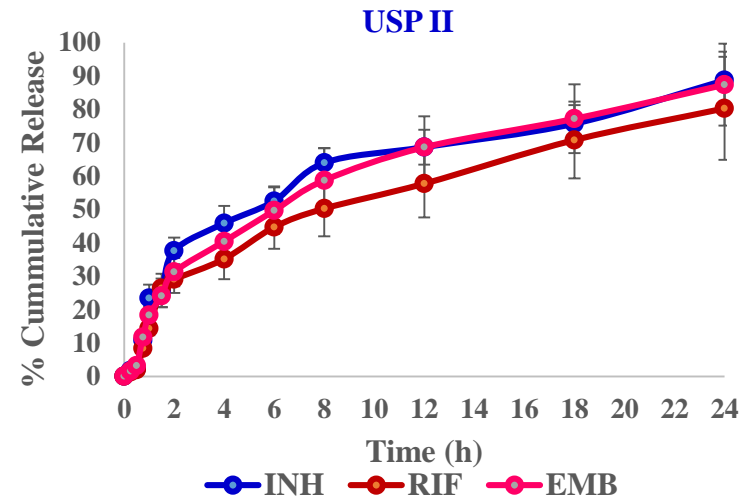
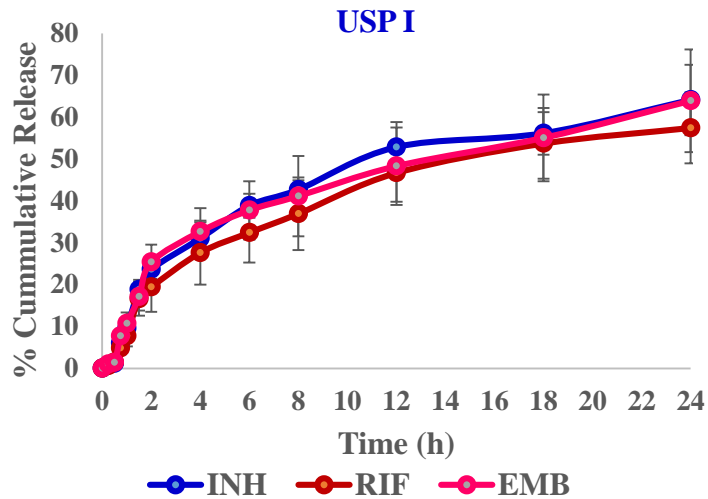
DISSOLUTION APPARATUS COMPARISON COMBINATION PARTICLES (SLF pH 7.4)



Standard deviation is very low with USP IV



DISSOLUTION APPARATUS COMPARISON COMBINATION PARTICLES (ALF pH 4.5)



Standard
deviation
is very
low with
USP IV



SUMMARY

- **USP IV WITH DIALYSIS CELL PRACTICAL APPROACH**
 - DISPOSABLE DIALYSIS CELLS OVERCOME OPERATIONAL DIFFICULTIES OF THE DIALYSIS CELL
 - SINK CONDITIONS CAN BE MAINTAINED
 - DISCRIMINATORY DISSOLUTION METHODS POSSIBLE
 - ADAPTABLE TO RANGE OF NANOFORMULATIONS



IDEAL DISSOLUTION TEST

- REPRODUCIBLE
- ROBUST
- PHYSIOLOGICALLY RELEVANT
- CONVENIENT **WITH THE FLOATALYZER**
- EASY TO USE
- DISCRIMINATORY
- MAINTAIN SINK CONDITION
- ADAPTABLE TO MANY **NANO-** FORMULATIONS



PROF. DEVARAJAN'S RESEARCH GROUP





ACKNOWLEDGEMENTS

- SOTAX INDIA PVT. LTD., FOR GENEROUS SUPPORT WITH USP IV
- Amit Lokhande



THANK YOU